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Biodegradation and Carbon Adsorption Carcinogenic and Hazardous Organic Compounds

IIT Research Inst.
Chicago, IL

Prepared for

Municipal Environmental Research Lab.
Cincinnati, OH

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16. ABSTRACT

This research program was conducted to determine the capability of biological treatment and activated carbon adsorption to remove chemical carcinogens and other hazardous organic compounds from water and wastewater. Compounds studied were

- benzidine
- 3,3'-dichlorobenzidine
- benzo[a]pyrene
- 2-acetyl-aminofluorene
- 4-aminobiphenyl
- 4-dimethylaminoazobenzene
- 4-nitrobiphenyl
- benzo(g,h,i)perylene
- dibenzo(a,h)anthracene
- benzo(k)fluoranthene
- 3,4-benzofluoranthene, and

All of the compounds tested exhibited some degree of biological degradation. Carbon adsorption was also effective in removing the compounds from aqueous solution. Large polynuclear aromatic compounds exhibited reduced adsorption capacities due to exclusion from the small diameter pores of the carbon.

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by

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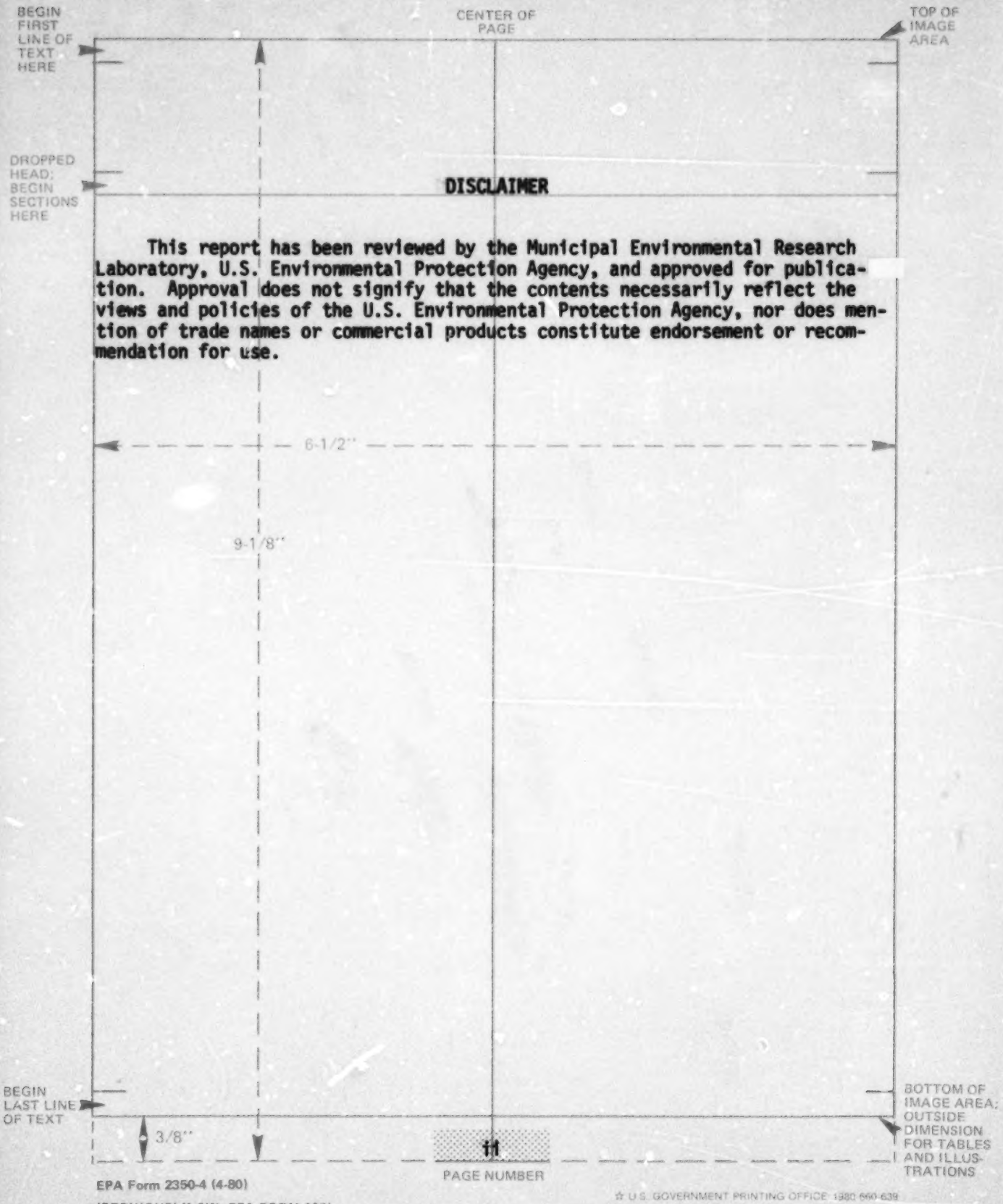
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FOREWORD

The U.S. Environmental Protection Agency was created because of increasing public and government concern about the dangers of pollution to the people. Noxious air, foul water, and spoiled land are tragic testimonies to the deterioration of our natural environment. The complexity of that environment and the interplay of its components require a concentrated and integrated attack on the problem.

Research and development is that necessary first step in problem solution; it involves defining the problem, measuring its impact, and searching for solutions. The Municipal Environmental Research Laboratory develops new and improved technology and systems to prevent, treat, and manage wastewater and solid and hazardous waste pollutant discharges from municipal and community sources, to preserve and treat public drinking water supplies, and to minimize the adverse economic, social, health, and aesthetic effects of pollution. This publication is one of the products of that research and provides a most vital communications link between the researcher and the user community.

This report presents data indicating the feasibility of removing some eleven different carcinogenic or hazardous chemical compounds from water by carbon adsorption or by biodegradation.

Francis T. Mayo, Director
Municipal Environmental Research Laboratory

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ABSTRACT

Carbon adsorption isotherms and susceptibility to biological degradation have been determined for 11 organic compounds selected from EPA's Priority Pollutant List and OSHA's list of regulated carcinogens. These compounds were:

- benzidine
- 3,3'-dichlorobenzidine
- benzo[a]pyrene
- 2-acetyl-aminofluorene
- 4-aminobiphenyl
- 4-dimethylaminoazobenzene
- 4-nitrobiphenyl
- benzo(g,h,i)perylene
- dibenzo(a,h)anthracene
- benzo(k)fluoranthene
- 3,4-benzofluoranthene

Special laboratory procedures were developed to deal with the low solubility of some of the compounds and with the significant degree of adsorption on glass surfaces.

This report was submitted in fulfillment of Contract 68-03-2834 by IIT Research Institute under the sponsorship of the U.S. Environmental Protection Agency. This report covers the period August 1979 to August 1980, and work was completed as of September 1980.

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IIT Research Institute (IITRI) gratefully acknowledges the cooperation of the National Cancer Institute, who provided some of the chemical carcinogens from their repository, and the advice and assistance of R. A. Dobbs, EPA Program Officer, who provided many valuable suggestions as to procedures and data presentation.

Victor Ivanuski and William Stepp, of the IITRI staff, conducted the major portion of the experimental work. Suellen Schulthesz assisted in the analysis of samples from the biological degradation studies.

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SECTION 1

INTRODUCTION

The work reported here is a continuation of the treatability studies conducted at IIT Research Institute (IITRI) in 1979 under Contract CI-68-03-2559 and reported in EPA 600/2-79-097, August 1979.[1]

These studies are part of the effort of the U.S. Environmental Protection Agency to develop treatment processes to remove toxic organic compounds from water and wastewater. The goal of this project was to develop treatability information for selected compounds known to be either highly toxic or carcinogenic. This work is supplementary to the treatability studies being conducted in the Municipal Environmental Research Laboratory.

Two treatment techniques--carbon adsorption and biological degradation--were used to evaluate the treatability of the following compounds:

- benzidine
- 3,3'-dichlorobenzidine
- benzo[a]pyrene
- 2-acetyl-aminofluorene
- 4-aminobiphenyl
- 4-dimethylaminoazobenzene
- 4-nitrobiphenyl
- benzo(g,h,i)perylene
- dibenzo(a,h)anthracene
- benzo(k)fluoranthene
- 3,4-benzofluoranthene

Carbon adsorption was studied using distilled water doped with the compound of interest and a commercially available activated carbon. The biological degradation studies used a static test procedure with a standard nutrient and biological seed obtained from a wastewater treatment plant serving a predominantly residential area.

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SECTION 2

RECOMMENDATIONS

These treatability studies should be continued and expanded to include treatment of combinations of compounds found in municipal wastewater. The previous study included ozone oxidation as a treatment technique; compounds studied here should also be subjected to this procedure.

Immediate future efforts should be directed toward determining the treatability of:

- 2,3,7,8-tetrachlorodibenzo-*p*-dioxin
- β-propiolactone
- ethyleneimine
- endeno(1,2,3,c,d)pyrene

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SECTION 3

MATERIALS AND METHODS

SELECTION OF COMPOUNDS TO BE STUDIED

The compounds studied during this program were selected from a list of 129 priority pollutants and from OSHA's list of regulated carcinogens. The laboratories at IITRI designed for the handling of toxic and hazardous materials permitted studies of these compounds to supplement the more extensive studies being conducted at the Municipal Environmental Research Laboratory.

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CARBON ADSORPTION

A standard experimental procedure for the determination of the carbon adsorption isotherms has been developed by MERL[2] and was used in these studies whenever feasible. This procedure uses granular activated carbon which has been ball milled to less than 325 mesh. Varying amounts of the pulverized activated carbon were added to one-liter aliquots of the doped distilled water and stirred for at least 2 hours to permit the carbon to attain equilibrium. The carbon was removed by filtration and the filtrate was analyzed for the compound.

The low water solubility and the tendency to adsorb on the glass containers required modification of this procedure; these modifications are discussed in the following section on experimental procedures.

Data have been presented as Freundlich adsorption isotherms using a standard format developed by MERL.[2] The Freundlich adsorption isotherm can be expressed as:

$$\frac{X}{M} = KC_f^{1/n}$$

where $X = C_o - C_f$

C_o = amount of organic in untreated solution

C_f = amount of organic in treated solution

M = weight of adsorbent (carbon)

K = empirical constant

1/n = empirical constant

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K is the X/M intercept of the plot of the isotherm at C_f = 1 and 1/n is the slope of the line on logarithmic paper.

BIOLOGICAL DEGRADATION

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All biological degradation studies were conducted using the static screening test reported by Bunch and Chambers.[2] The scale of the test was increased from 200 ml to 2000 ml to provide a sample large enough for analysis of the very low concentrations. Bunch and Chambers seeded a nutrient/organic solution with sewage and allowed the bacteria to act on the nutrient and organic for seven days. After analyzing for the organic, they used a 10 percent aliquot of the seven-day mixture to seed a fresh nutrient/organic mixture. This procedure was repeated until 28 test-days had been completed.

In the previous study, those compounds that resisted degradation during the static test were studied using a continuous flow biological reactor. In these studies, all the compounds were significantly decomposed by the static biological screening test, and no continuous biological degradation studies were conducted.

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SECTION 4

EXPERIMENTAL PROCEDURES

GENERAL PROCEDURES

Before beginning work on the program, all project personnel attended a course on *The Safe Handling of Chemical Carcinogens*, sponsored by the National Cancer Institute, Office of Research Safety.[3] In addition, the staff was instructed in specific procedures to be used for each compound. Standard solutions were prepared in glove boxes, and protective clothing (gloves and disposable laboratory coats) was used during handling of dilute solutions. All staff members involved in the laboratory phases of the program were under medical surveillance. (No medical incidents occurred during the program.)

Laboratory wastes were incinerated in equipment which serves the laboratories using chemical carcinogens.

Quality control procedures were maintained to ensure clean, contaminant-free water and glassware. Analytical instruments were calibrated against the manufacturer's specifications at the start of the effort and routinely monitored throughout the program. Stability of standard solutions was monitored, and fresh standards were made up weekly.

Adsorption capacity of the activated carbon was checked periodically throughout the program by determining the adsorption isotherm for a standard solution of para-nitroaniline. No significant change was observed.

The mineralized water and nutrient used in the biological degradation studies was the same as used for the previous program; preparation procedures are given in Appendix B.

CARBON ADSORPTION

Carbon adsorption isotherms were determined using a commercially available granular activated carbon (GAC) Filtrasorb 400. The GAC was ball milled to improve the rate of adsorption and to permit addition in small amounts. Since this work was supplementary to the studies of MERL, the adsorptive capacity of this lot of carbon was compared to the adsorptive capacity of the MERL carbon by determining the adsorption isotherm using para-nitroaniline.

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Two adsorption isotherms were determined; one with carbon which had been ball milled and passed through 200 mesh screen and the second isotherm with this same carbon except that the -400 mesh fraction had been removed.

The carbon which had been screened to -200 mesh contained a surprising amount of -400 mesh fines, almost 45 percent.

The para-nitroaniline isotherm obtained using the -200 mesh carbon (which contained the fines) was determined. Values of K = 160, 1/n = 0.20, with a correlation coefficient of 0.99 for 12 data points, were determined.

This carbon was then screened to remove the -400 mesh material, and the isotherm was determined for para-nitroaniline. Values of K = 148, 1/n = 0.22, with a correlation coefficient of 0.97 for 12 data points, were obtained. This compares with the MERL isotherm of K = 140 and 1/n = 0.27. The isotherm plots are shown in Figure 1.

The agreement between the MERL and IITRI isotherms was good; it was assumed that the GAC used at MERL and IITRI would give comparable results on other compounds. This -200 + 400 mesh GAC was used for the program.

Two somewhat differing procedures were required to determine the carbon adsorption isotherms. The first procedure was identical to the procedure used in the previous work at IITRI and MERL. The second procedure was developed to cope with the problems presented by the low solubility, adsorption on glass, and analytical requirements of some of the compounds.

In the first procedure, a series of eight 1-liter, glass-stoppered reagent bottles was used for each carbon adsorption isotherm determination. Automatic pipettes were used to charge the clean, well-rinsed bottles with a known amount of an aqueous suspension of activated carbon of known concentration. The bottles were filled with the doped water to the bottom of the stopper to avoid air space over the solution. The suspensions were agitated with magnetic stirrers for at least 2 hours before removing the sample for analysis.

Six carbon adsorption samples were obtained from the eight bottles. One bottle served as a control and did not receive any carbon nor was it filtered. A second bottle did not receive carbon but was filtered. These two samples served as an analytical control for assessing the effect of filtration in removing the organic from the water. The filtered sample indicated whether or not the organic completely dissolved or remained as finely divided undissolved particles.

This procedure and control technique indicated that some compounds, notably benzo[a]pyrene, adsorbed on the glass surface of the reagent bottle, the surface of the filter bottle, the surface of the glass fiber surface, the surface of the sample bottle, and the surface of the cell used to determine fluorescence. In addition, the techniques used to saturate the solution resulted in undissolved platelets of the benzo[a]pyrene. These problems required the development of a second procedure.

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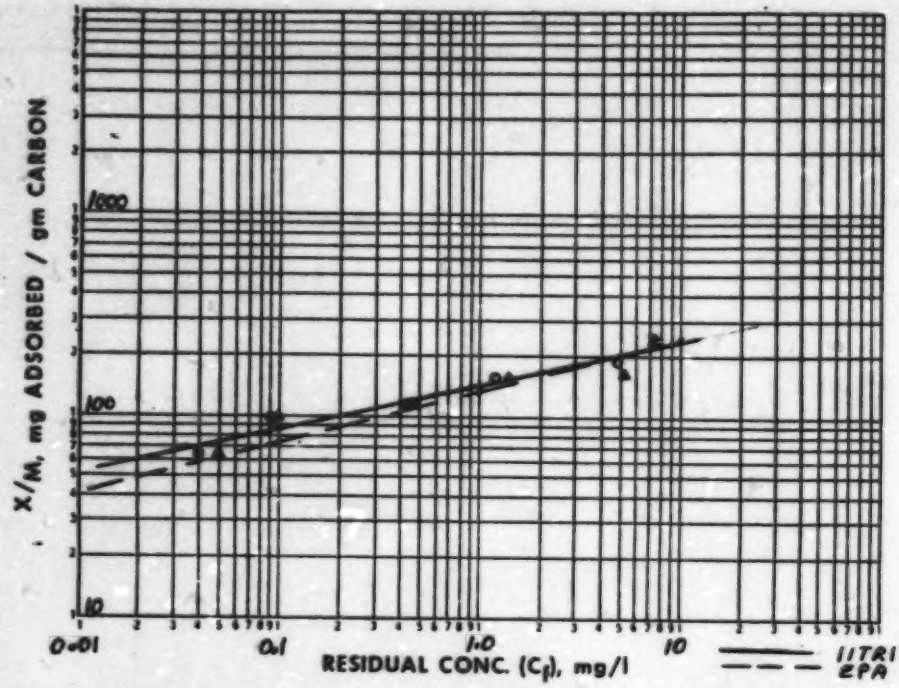

CARBON DOSE mg/l	pH= C_f	$C_o-C_f=X$	X/M	○ pH=7.0 C_f	$C_o-C_f=X$	X/M	△ pH=7.0 C_f	$C_o-C_f=X$	X/M
0				9.75			9.75		
10				7.28	2.47	247	7.28	2.47	247
25				4.85	4.90	196	5.35	4.40	176
50				1.20	8.55	171	1.32	8.43	168
75				0.18	9.32	124	0.18	9.27	124
100				.09	9.66	97	0.10	9.65	97
150				.09	9.71	65	.05	9.70	65

Figure 1. Adsorption isotherm for para-nitroaniline.

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STRUCTURE:

FORMULA: $C_6 H_6 N_2 O_2$ **MOL. WT.** 138.13

FREUNDLICH PARAMETERS	pH = 7.0		
	all data pooled		
K	148		
1/n	0.225		
Corr. Coef. r	0.972		
INITIAL CONC. mg/l	ADSORPTION CAPACITY, mg/gm		
10	248		
1.0	148		
0.1	88		

CARBON DOSES REQUIRED TO ACHIEVE INDICATED CHANGE IN CONCENTRATION[a]

	C_f, mg/l			
C_o, mg/l	1.0	0.1	0.01	0.001
10	61.0	112	190	320
1.0		10	19	32
0.1			1.7	3.2
0.01				0.3

(a) Carbon doses in mg/l at neutral pH.

REMARKS: These data for Filtrasorb 400; MERL did similar isotherm for Filtrasorb 300.

Agreement of IITRI and MERL data is good.

U.V. adsorption at 380 nm.

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Figure 1. (continued)

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The second procedure used to determine the carbon adsorption isotherm was much more complex and required much more time and effort. In this procedure, 3 liters of distilled water were placed in a 5-liter reagent bottle. The water was saturated and filtered by pumping out of the bottle through a column (1-in. in diameter by 18-in. long) of 1 mm glass beads coated with the organic compound of interest, and back to the container. The glass beads were coated by removing a volatile solvent from a mixture of beads and solution using a rotary evaporator. The top of the glass bead column was packed with glass wool to act as a filter. A strong light beam was used to examine the solution for particles of the undissolved organic. Chemical analysis was used to monitor concentration and determine when an equilibrium concentration had been established. Generally a circulation rate of 60 ml per minute was used and the circulation continued for at least 4 hours.

The low solubility of the compound required the addition of very small amounts of GAC, about 1 mg/l. It was found that allowing the carbon to settle for one hour before withdrawing a sample resulted in a virtually carbon-free sample and avoided the problem of loss of compound to glass surfaces during filtration.

Adsorption of the compound on the surface of the sample container was avoided by using an alcohol solution. Exactly 40 ml of methyl alcohol was put into a cleaned, thoroughly rinsed 100 ml volumetric flask. A sample of 60 ml was withdrawn from the 3 liters of treated solution using a 100-ml pipette which had been rinsed with the treated solution. The 40 percent alcohol solution minimized adsorption of the compound on the surfaces of the sample container and the analytical cell. Analytical standards were also 40 percent methyl alcohol.

BIOLOGICAL DEGRADATION

The biological degradation of the compounds was determined by the method described by Tabak.[4]

Approximately 1 percent of the organic compound of interest was dissolved in an emulsifier (Emulphor EL-620, GAF Corporation) and added to the bacterial suspension at concentration levels of 1-2 ppm.

The suspension was analyzed as soon as it was prepared and after the 7-day incubation period. The analytical procedure generally involved serial extraction of a 1-liter sample with 70, 70, and 70 ml of methylene chloride. The extract was then concentrated in a Kuderna-Danish (KD) apparatus fitted with a 3-ball macro-Snyder column and a calibrated receiver tube and methanol was added. The sample was then evaporated to a predetermined volume.

Samples were analyzed on a Waters Model 244 ALC/GPC liquid chromatograph equipped with a Model 660 solvent programmer for gradient elution and a Schoeffel HS 870 ultraviolet absorption detector.

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SECTION 5

RESULTS AND DISCUSSION

The compounds exhibited a range of treatability both by carbon adsorption and biological degradation. The treatability of each compound by both processes is discussed in the following sections.

CARBON ADSORPTION

Data for the carbon adsorption are presented in Table 1. It should be noted that the intercept, K, given in this table is calculated at a concentration of 1 mg/l. Most of the compounds were soluble only at a level of 1 ppb or less; the calculated intercept is presented for the purpose of comparing relative adsorption of the compounds.

BIOLOGICAL DEGRADATION

All of the compounds studied exhibited some degree of degradation in the static biological test as shown in Table 2.

It is of interest to note that 4-nitrobiphenyl was reduced to 4-aminobiphenyl, and then the 4-aminobiphenyl was biologically degraded. This led to some confusion, since in the initial test, several groups of compounds were studied in a single vessel. One group contained both 4-nitrobiphenyl and 4-aminobiphenyl.

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TABLE 1. FREUNDLICH PARAMETERS AND CAPACITY OF GAC

Compound	Mol wt	pH	Freundlich parameters K	1/n	Correl. coeff.	Number of data points	Calculated mg carbon per liter to reduce from 1.0 mg/l to 0.1 mg/l
benzidine	184	9.1/7.1	215	0.16	0.96	16	6
3,3'-dichlorobenzidine	253	7.2	308	0.19	0.97	8	5
benzo[a]pyrene	252	7.1	33.6	0.44	0.90	6	0.4
2-acetyl-aminofluorene	222	7.1	318	0.12	0.94	7	3.8
4-aminobiphenyl	169	7.2	198	0.26	0.96	9	8
4-dimethylaminoazobenzene	225	7.0	250	0.24	0.96	8	6.2
4-nitrobiphenyl	199	7.0	370	0.27	0.98	6	4.5
benzo(g,h,i)perylene	276	7.0	10.6	0.36	0.92	8	197
dibenzo(a,h)anthracene	278	7.1	70.2	0.76	0.97	8	73
benzo(k)fluoranthene	252	7.1	183	0.67	0.98	8	18
3,4-benzofluoranthene	252	7.0	55.5	0.37	0.95	8	0.5

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6-1/2"

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BOTTOM OF IMAGE AREA. OUTSIDE DIMENSION FOR TABLES AND ILLUSTRATIONS

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9-1

TABLE 2. BIOLOGICAL DEGRADATION TESTS*

Culture	Benzo[a]-pyrene	2-Acetyl...no-fluorene	4-Amino-biphenyl	4-Dimethylamino-azobenzene	4-Nitro-biphenyl	Benzo(g,h,i)-perylene	Dibenzo(a,h)-anthracene	Benzo(k)-fluoranthene
Original Culture								
Doped	1.02	2.20	2.01	0.29	2.00	1.00	1.10	1.22, 1.09
7-Day	0.34	1.94	1.09	trace	0.05	0.30	0.20	0.60, 0.34
1st Sub-Culture								
Doped	1.05	2.27		0.29	2.03	1.03	1.18	1.28, 1.12
7-Day	0.46	1.89		N.D.**	N.D.	1.08	0.21	1.30, 0.47
2nd Sub-Culture								
Doped	1.10	2.24		0.29		1.22	1.14	1.52, 1.17
7-Day	0.38	1.76		N.D.		0.79	0.21	0.60, 0.39
3rd Sub-Culture								
Doped	1.14	2.38		0.29		1.22	1.16	1.52, 1.21
7-Day	0.41	2.21		N.D.		0.34	0.29	0.60, 0.43

* All values in mg/l.
**N.D. - none detected

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EPA-287 (Cin.)
(4-76)

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REFERENCES

1. Fochtman, E.G., and W. Eisenberg. *Treatability of Carcinogenic and Other Hazardous Organic Compounds.* EPA 600/2-79-097, August 1979.

2. Dobbs, R.A., and J.M. Cohen. *Carbon Adsorption Isotherms for Toxic Organics.* EPA 600/8-80-023, April 1980, p. 309.

3. Remaly, R.F. Course presented by IIT Research Institute, Chicago, Ill., supported by the National Cancer Institute, Office of Research Safety, Contract NO1-CO-95425.

4. Tabak, H.H. *Static Procedure for Biodegradability Determination.* Draft, May 1979, EPA, MERL, Cincinnati, Ohio.

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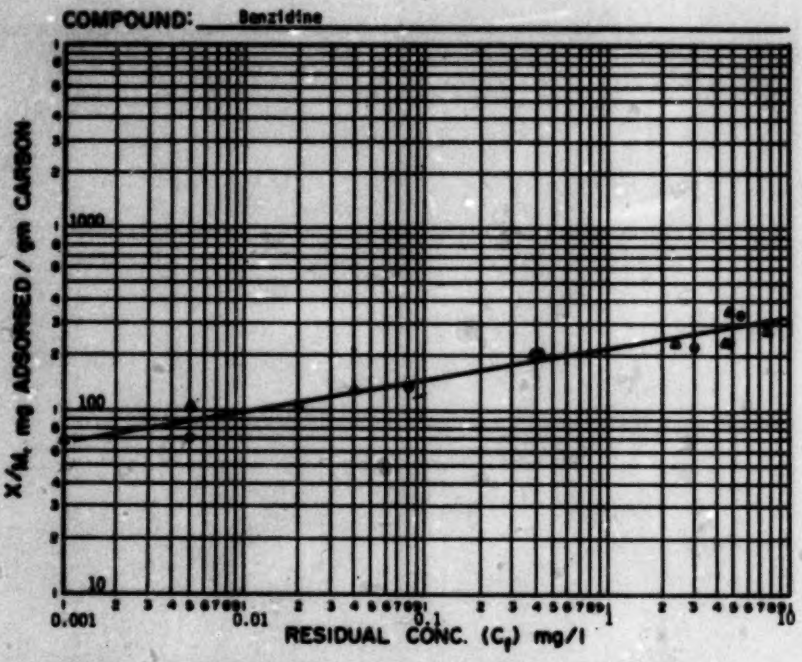
APPENDIX A

CARBON ADSORPTION DATA



CARBON DOSE mg/l	● pH = 7.1			▲ pH = 9.1			pH =		
	C_f	$C_o-C_f=X$	X/M	C_f	$C_o-C_f=X$	X/M	C_f	$C_o-C_f=X$	X/M
0	10.80			10.50					
10	7.80	3.00	300	7.70	2.80	280			
16	5.40	5.40	337	4.80	5.70	356			
25	4.88	5.92	237	4.60	5.90	236			
35	3.00	7.80	223	2.30	8.20	234			
50	0.41	10.39	208	0.39	10.11	202			
75	0.08	10.72	143	0.04	10.46	139			
100	0.02	10.78	107	0.01	10.49	105			
150	0.01	10.79	72	0.001	10.499	70			

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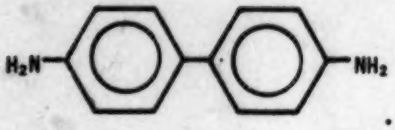
COMPOUND: Benzidine/Filtrasorb 400

STRUCTURE:



FORMULA: $NH_2(C_6H_4)_2NH_2$ **MOL. WT.** 184.23

FREUNDLICH PARAMETERS	pH		
	pH pooled		
K	215		
1/n	0.16		
Corr. Coef. r	0.96		
INITIAL CONC. mg/l	ADSORPTION CAPACITY, mg/gm		
10	313		
1.0	215		
0.1	148		
0.01	101		

CARBON DOSES REQUIRED TO ACHIEVE INDICATED CHANGE IN CONCENTRATION[a]

	C_f, mg/l			
C_o, mg/l	1.0	0.1	0.01	0.001
10	43	71	109	165
1.0	--	6	11	16
0.1	--	--	1	1.6
0.01	--	--	--	0.1^+

(a) Carbon doses in mg/l at neutral pH.

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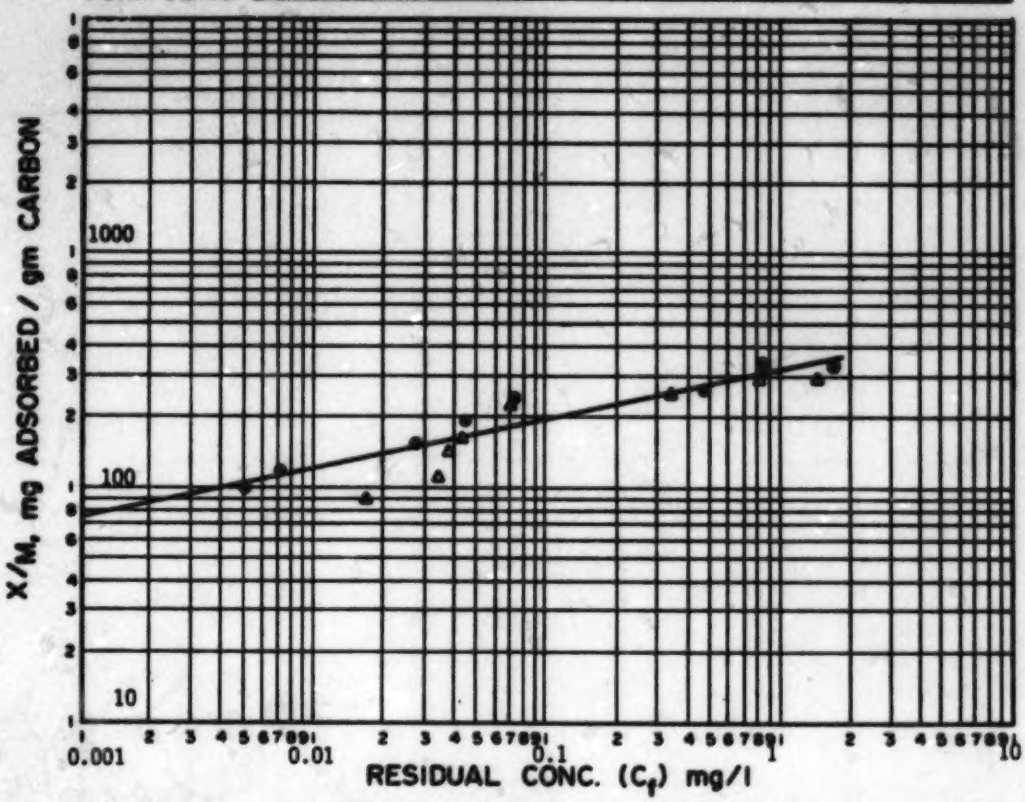
COMPOUND: 3,3-Dichlorobenzidine



CARBON DOSE mg/l	● pH = 7.2			▲ pH = 9.1			pH =		
	C_f	$C_o-C_f=X$	X/M	C_f	$C_o-C_f=X$	X/M	C_f	$C_o-C_f=X$	X/M
0	2.51			2.28					
2.5	1.70	0.81	324	1.55	0.73	292			
5	0.83	1.68	336	0.81	1.47	294			
7.5	0.46	2.05	273	0.33	1.95	260			
10	0.073	2.44	244	0.070	2.210	221			
12.5	0.044	2.466	197	0.042	2.238	179			
15	0.028	2.482	165	0.038	2.242	149			
20	0.008	2.502	125	0.034	2.246	112			
25	0	2.505	100	0.017	2.263	90			

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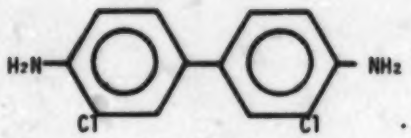
COMPOUND: 3,3-Dichlorobenzidine

STRUCTURE:



FORMULA: $C_{12}H_{10}Cl_2N_2$ **MOL. WT.** 253.13

FREUNDLICH PARAMETERS	pH		
	7.2	9.1	All Data Pooled
K	308	310	304
1/n	0.19	0.24	0.20
Corr. Coef. r	0.97	0.89	0.92
INITIAL CONC. mg/l	ADSORPTION CAPACITY, mg/gm		
1.0	308	310	304
0.1	200	179	189
0.01	130	103	118
0.001	84	60	74

CARBON DOSES REQUIRED TO ACHIEVE INDICATED CHANGE IN CONCENTRATION[a]

	C_f, mg/l			
C_o, mg/l	1.0	0.1	0.01	0.001
10	29.0	55.3	96.6	167.4
1.0	--	5.0	9.6	16.7
0.1	--	--	0.9	1.7
0.01	--	--	--	0.2

(a) Carbon doses in mg/l at neutral pH.

REMARKS:

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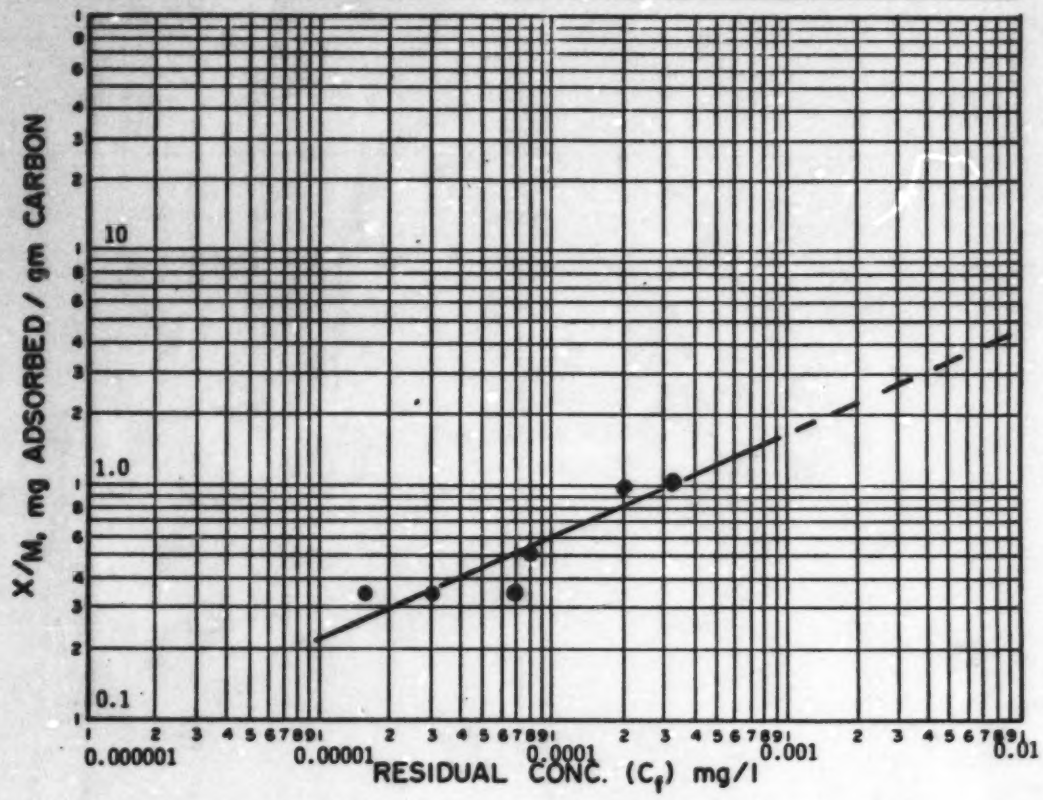
COMPOUND: Benzo[a]pyrene



DATE	Carbon Dose mg/l	C_o ppm	C_f ppm	X C_o-C_f	X/M mg/g	
10/10	1.2	0.00160	0.00032	0.00128	1.067	
10/11	1.2	0.00138	0.00020	0.00118	0.983	
10/12	3.89	0.00140	0.000017	0.00138	0.355	
10/17	3.89	0.00205	0.00008	0.00197	0.506	
10/23	4.0	0.00147	0.00007	0.00140	0.350	
10/24	4.0	0.00135	0.00003	0.00132	0.330	

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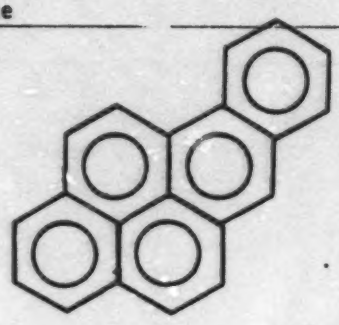
COMPOUND: Benzo[a]pyrene

STRUCTURE:



FORMULA: $C_{20}H_{12}$ **MOL. WT.** 252.30

FREUNDLICH PARAMETERS	pH		
	7.1		
K	33.63		
1/n	0.44		
Corr. Coef. r	0.90		
INITIAL CONC. mg/l	ADSORPTION CAPACITY, mg/gm		
0.010	1.638		
0.0001	0.598		
0.00001	0.218		
0.000001	0.080		

CARBON DOSES REQUIRED TO ACHIEVE INDICATED CHANGE IN CONCENTRATION[a]

	C_f, mg/l			
Co, mg/l	0.0001	0.00001	0.000001	0.0000001
0.001	1.505	4.533	12.527	34.335
0.0001	--	0.412	1.241	3.430
0.00001	--	--	0.113	0.340
0.000001	--	--	--	0.031

(a) Carbon doses in mg/l at neutral pH.

REMARKS:

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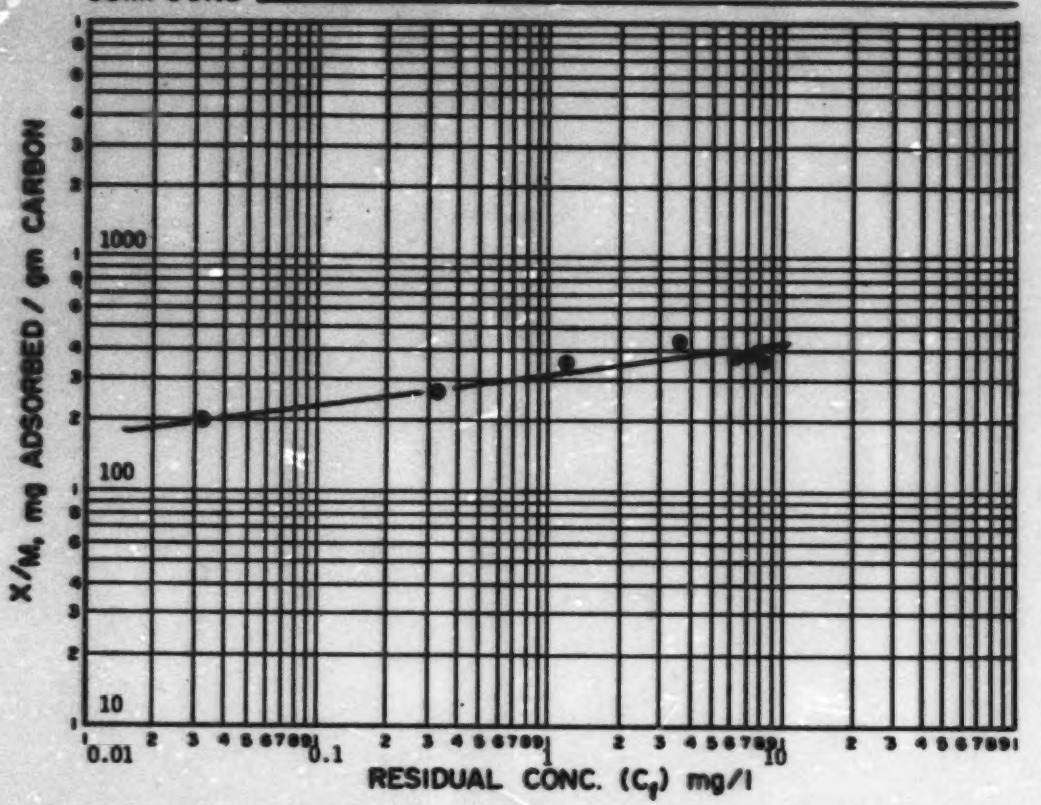
COMPOUND: 2-Acetyl-aminofluorene



	pH = 7.1			pH =			pH =		
CARBON DOSE mg/l	C_f	$C_o-C_f=X$	X/M	C_f	$C_o-C_f=X$	X/M	C_f	$C_o-C_f=X$	X/M
0	10.02								
5.0	8.20	1.82	364						
7.5	7.05	2.97	396						
10	6.20	3.82	382						
15	3.55	6.47	431						
25	1.28	8.74	350						
35	0.32	9.70	277						
50	0.032	9.99	200						

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BOTTOM OF IMAGE AREA; OUTSIDE DIMENSION FOR TABLES AND ILLUSTRATIONS

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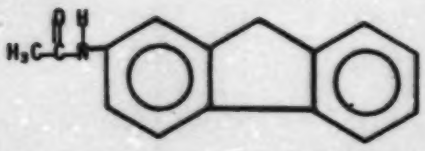
COMPOUND: 2-Acetyl-aminofluorene

STRUCTURE:



FORMULA: $C_{15}H_{13}ON$ **MOL. WT.** 222.28

FREUNDLICH PARAMETERS	pH		
	7.1		
K	318		
1/n	0.12		
Corr. Coef. r	0.94		
INITIAL CONC. mg/l	ADSORPTION CAPACITY, mg/gm		
10	421		
1.0	318		
0.1	240		
0.01	181		

CARBON DOSES REQUIRED TO ACHIEVE INDICATED CHANGE IN CONCENTRATION(a)

	C_f, mg/l			
C_o, mg/l	1.0	0.1	0.01	0.001
10	28.3	41.3	55.1	73.1
1.0	--	3.8	5.5	7.3
0.1	--	--	0.49	0.72
0.01	--	--	--	0.06

(a) Carbon doses in mg/l at neutral pH.

REMARKS: Measured at 270 nm wavelength.
Solubility >10 ppm.

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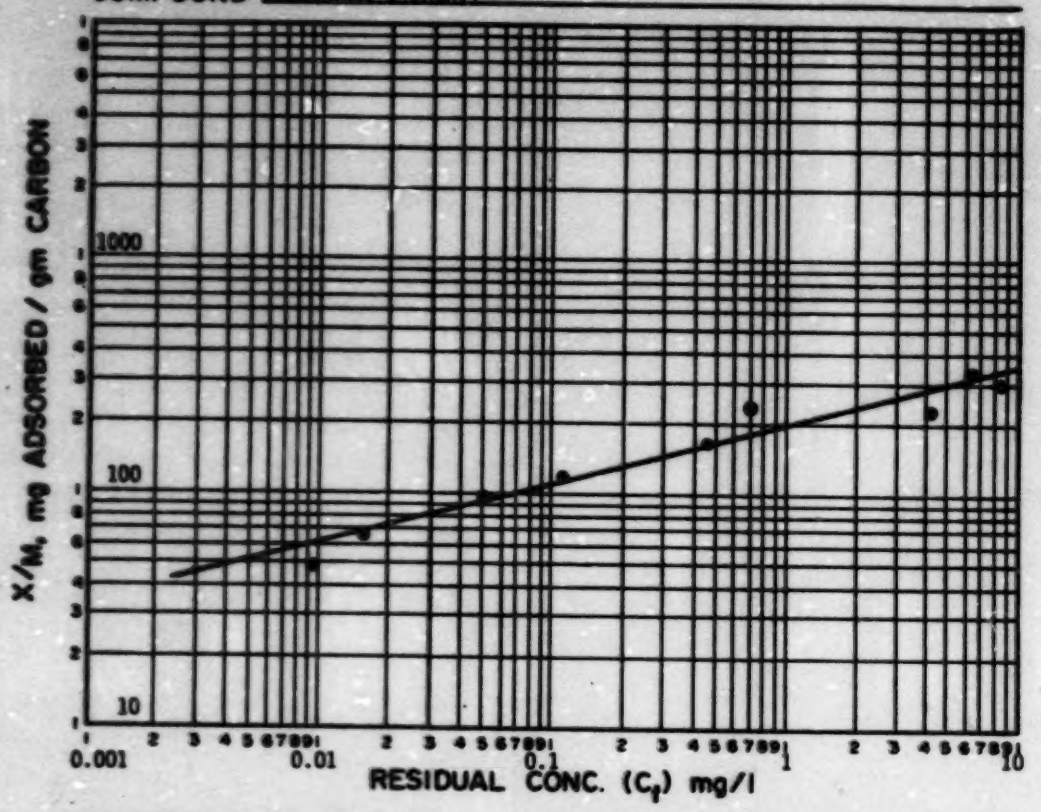
COMPOUND: 4-Amino Biphenyl



CARBON DOSE mg/l	● pH = 7.2			pH =			pH =		
	C_f	$C_o-C_f=X$	X/M	C_f	$C_o-C_f=X$	X/M	C_f	$C_o-C_f=X$	X/M
0	9.80	9.80							
5	8.30	1.50	300						
10	6.50	3.30	330						
25	4.20	5.60	224						
37.5	0.70	9.10	243						
50	0.46	9.34	187						
75	0.115	9.69	129						
100	0.05	9.75	98						
150	0.017	9.78	65						
200	>0.01	9.79	49						

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BOTTOM OF IMAGE AREA; OUTSIDE DIMENSION FOR TABLES AND ILLUSTRATIONS

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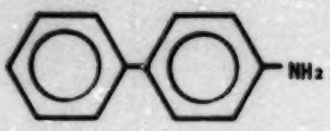
COMPOUND: 4-Amino Biphenyl

STRUCTURE:



FORMULA: $C_{12}H_{11}N$ **MOL. WT.** 169.12

FREUNDLICH PARAMETERS	pH		
	7.2		
K	198		
1/n	0.26		
Corr. Coef. r	0.96		
INITIAL CONC. mg/l	ADSORPTION CAPACITY, mg/gm		
10	358		
1.0	198		
0.1	110		
0.01	61		

CARBON DOSES REQUIRED TO ACHIEVE INDICATED CHANGE IN CONCENTRATION[a]

	C_f, mg/l			
Co, mg/l	1.0	0.1	0.01	0.001
10	45.5	90.4	165	298
1.0	--	8	16	30
0.1	--	--	1	3
0.01	--	--	--	0.3

(a) Carbon doses in mg/l at neutral pH.

REMARKS:

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EPA-287 (Cin.)
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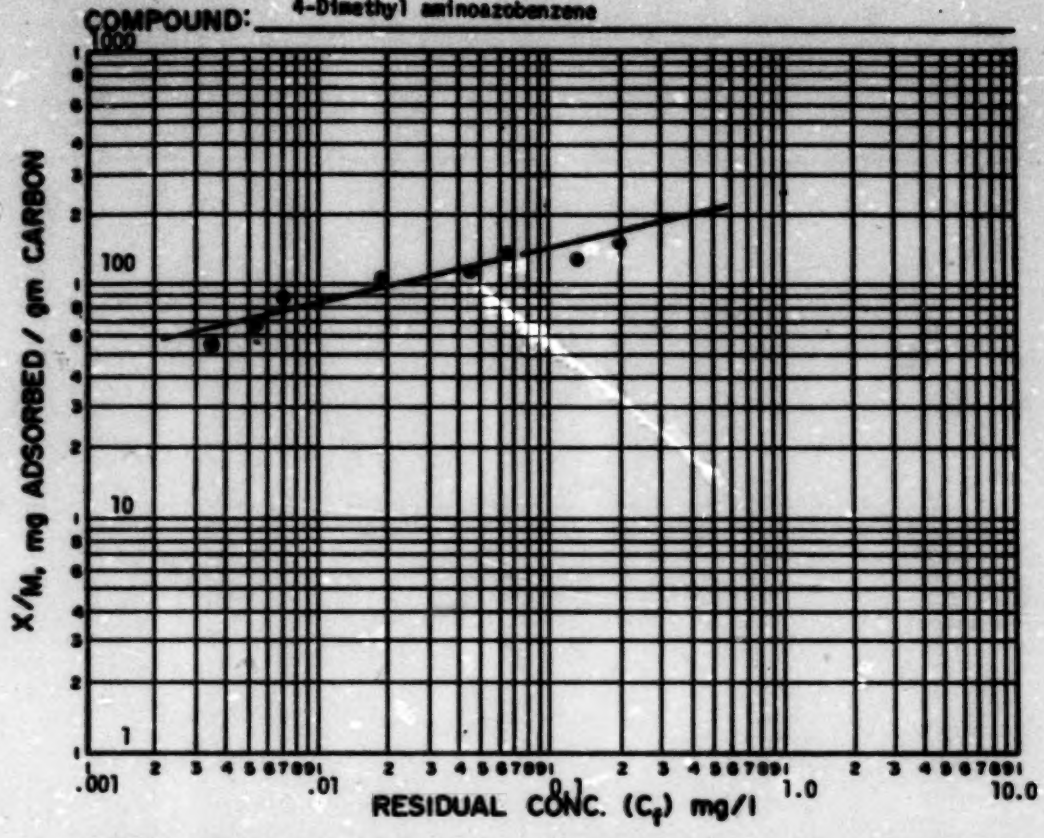
COMPOUND: 4-Dimethyl aminoazobenzene



CARBON DOSE mg/l	pH = 7.0			pH =			pH =		
	C_f	$C_o-C_f=X$	X/M	C_f	$C_o-C_f=X$	X/M	C_f	$C_o-C_f=X$	X/M
0	0.280								
0.5	0.198	0.082	164.0						
1.0	0.138	0.142	142.0						
1.5	0.065	0.215	143.3						
2.0	0.045	0.235	117.5						
2.5	0.019	0.261	104.4						
3.0	0.007	0.273	91.0						
4.0	0.005	0.275	68.7						
5.0	0.003	0.277	55.3						

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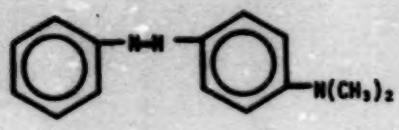
COMPOUND: 4-Dimethyl aminoazobenzene

STRUCTURE:



FORMULA: $C_{14}H_{15}N_3$ **MOL. WT.** 225.3

FREUNDLICH PARAMETERS	pH		
	7.0		
K	249		
1/n	0.24		
Corr. Coef. r	0.96		
INITIAL CONC. mg/l	ADSORPTION CAPACITY, mg/gm		
10	430		
1	249		
0.1	144		
0.01	83		

CARBON DOSES REQUIRED TO ACHIEVE INDICATED CHANGE IN CONCENTRATION[a]

	C_f, mg/l			
Co, mg/l	1	0.1	0.01	0.001
10	36.1	68.7	119.1	207.4
1	--	6.2	11.9	20.7
0.1	--	--	1.1	2.1
0.01	--	--	--	0.2

(a) Carbon doses in mg/l at neutral pH.

REMARKS: Measured at 450 nm wavelength.
Solubility <0.5 ppm.

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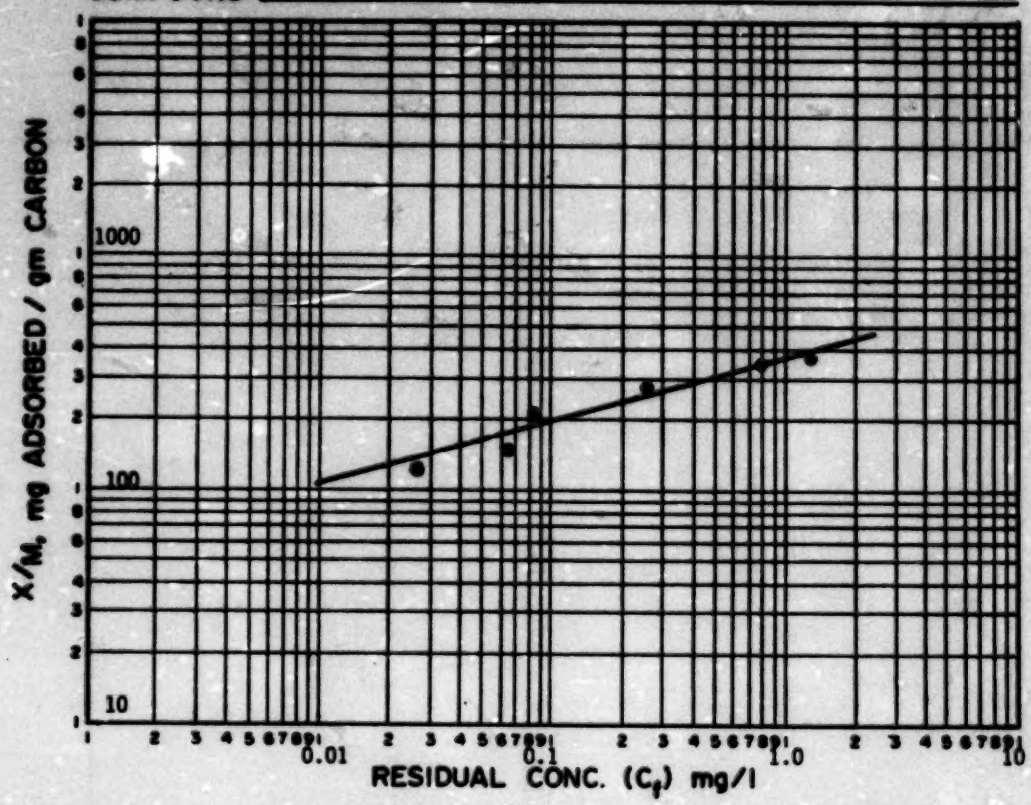
COMPOUND: 4-Nitrobiphenyl



CARBON DOSE mg/l	pH = 7.0			pH =			pH =		
	C_f	$C_o-C_f=X$	X/M	C_f	$C_o-C_f=X$	X/M	C_f	$C_o-C_f=X$	X/M
0	1.670								
1.0	1.30	0.370	370						
2.5	0.800	0.870	348						
5.0	0.260	1.410	282						
7.5	0.082	1.588	212						
10.0	0.064	1.606	161						
12.5	0.027	1.643	131						

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BOTTOM OF IMAGE AREA; OUTSIDE DIMENSION FOR TABLES AND ILLUSTRATIONS

EPA Form 2350-4 (4-80)

(PREVIOUSLY CIN. EPA FORM 287)

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COMPOUND: 4-Nitrobiphenyl

STRUCTURE:

FORMULA: $C_{12}H_9NO_2$ **MOL. WT.** 199.21

FREUNDLICH PARAMETERS	pH		
	7.0		
K	370		
1/n	0.27		
Corr. Coef. r	0.98		
INITIAL CONC. mg/l	ADSORPTION CAPACITY, mg/gm		
10	690		
1.0	370		
0.1	198		
0.01	106		

CARBON DOSES REQUIRED TO ACHIEVE INDICATED CHANGE IN CONCENTRATION[a]

	C_f, mg/l			
C_o, mg/l	1.0	0.1	0.01	0.001
10	24.35	49.97	94.07	175.64
1.0	--	4.54	9.32	17.55
0.1	--	--	0.85	1.74
0.01	--	--	--	0.16

(a) Carbon doses in mg/l at neutral pH.

REMARKS: UV analysis by adsorption at 325 Å.

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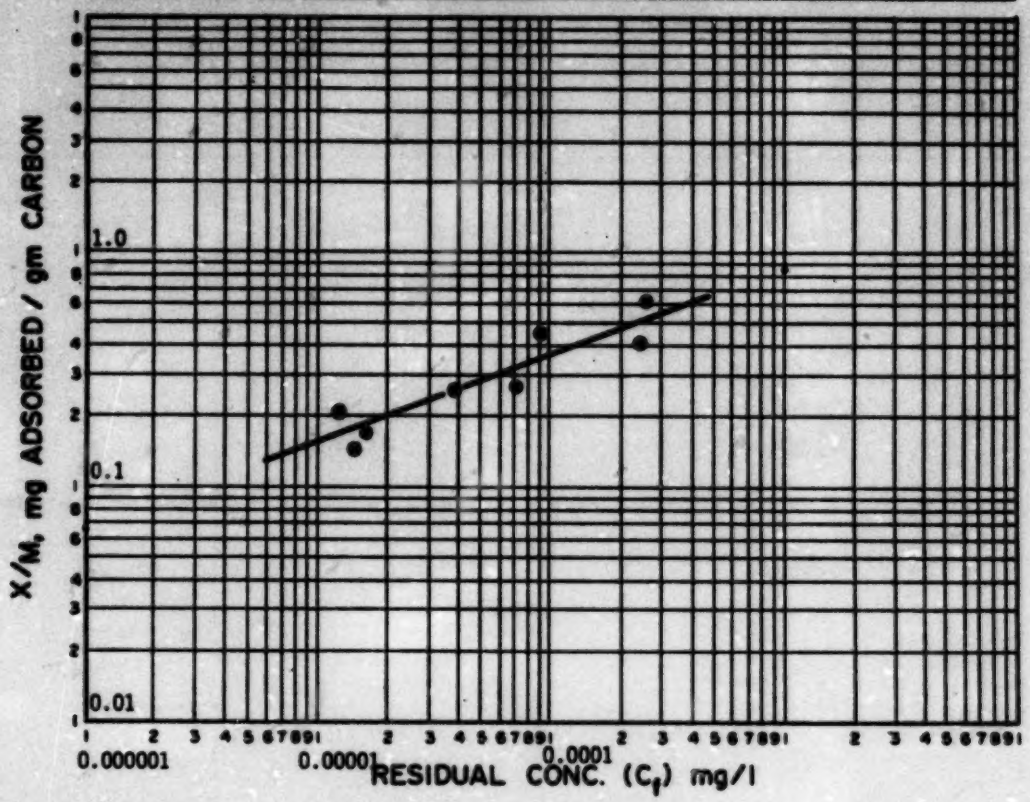
COMPOUND: Benzo(g,h,i)perylene



Date	C_o	M mg/l	C_f	X C_o-C_f	X/M	
010780A*	0.000353	0.1408	0.000267	0.000086	0.610	
010780B	0.000306	0.1458	0.000246	0.000060	0.410	
010880A*	0.000157	0.1429	0.000090	0.000067	0.469	
011180A*	0.000096	0.4478	0.000016	0.000080	0.179	
011180B	0.000087	0.4545	0.000015	0.000072	0.158	
011480A	0.000120	0.3077	0.000039	0.000081	0.263	
011480B	0.000076	0.3030	0.000013	0.000063	0.208	
011580A	0.000113	0.1563	0.000071	0.000042	0.269	

*Pumped overnight

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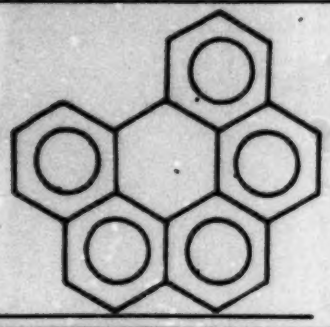
COMPOUND: Benzo(g,h,i)perylene

STRUCTURE:



FORMULA: $C_{22}H_{12}$ **MOL. WT.** 276.34

FREUNDLICH PARAMETERS	pH		
	7.0		
K	10.6		
1/n	0.36		
Corr. Coef. r	0.92		
INITIAL CONC. mg/l	**ADSORPTION CAPACITY, mg/gm**		
10	24.6		
1.0	10.6		
0.1	4.6		
0.01	2.0		

CARBON DOSES REQUIRED TO ACHIEVE INDICATED CHANGE IN CONCENTRATION[a]

C_f, mg/l

Co, mg/l	1.0	0.1	0.01	0.001
10	849	2165	5064	>10,000
1.0	--	197	502	1,174
0.1	--	--	46	116
0.01	--	--	--	10.6

(a) Carbon doses in mg/l at neutral pH.

REMARKS: Solubility in H_2O is less than 0.0003 mg/l.
Analysis by fluorescence, excitation 293 nm, emission 415 nm.

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EPA Fonn 2350-4 (4-80)

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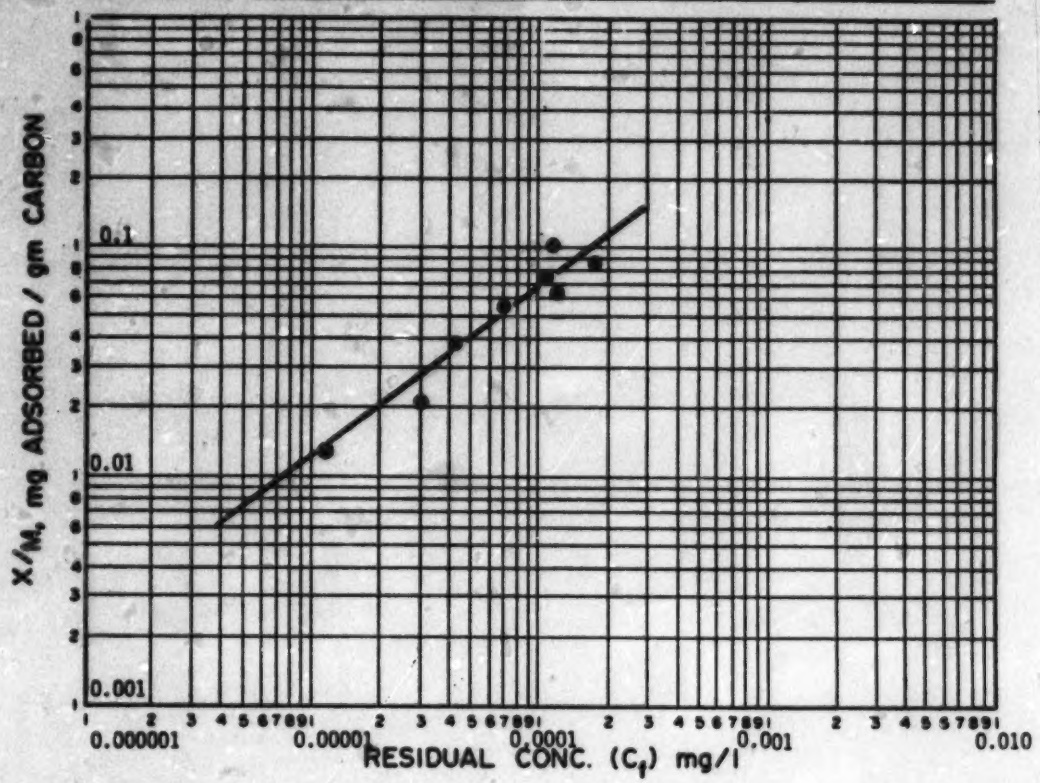
COMPOUND: Dibenzo(a,h)anthracene



DATE	Co mg/l[1]	mg/l Carbon	C_F mg/l	X C_o-C_F	X/M mg/g	
1204A*	0.000428	2.817	0.000182	0.000246	0.087	
1204B	0.000298	2.857	0.000114	0.000184	0.064	
1205A*	0.000185	7.463	0.000030	0.000155	0.021	
1205B	0.000112	6.944	0.000012	0.000100	0.014	
1206A*	0.000199	0.725	0.000126	0.000073	0.101	
1206B	0.000166	0.735	0.000109	0.000057	0.078	
1207A*	0.000133	2.344	0.000041	0.000092	0.039	
1207B	0.000246	3.175	0.000070	0.000176	0.055	

*Solution pumped overnight
[1]Value after 2 hr of air stirring

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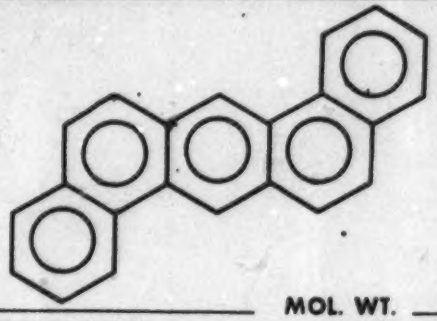
COMPOUND: Dibenzo(a,h)anthracene

STRUCTURE:



FORMULA: $C_{22}H_{14}$ **MOL. WT.** 278.33

FREUNDLICH PARAMETERS	pH		
	7.1		
K	70.21		
1/n	0.76		
Corr. Coef. r	0.97		
INITIAL CONC. mg/l	ADSORPTION CAPACITY, mg/gm		
10	399		
1.0	70.21		
0.1	12.33		
0.01	2.17		

CARBON DOSES REQUIRED TO ACHIEVE INDICATED CHANGE IN CONCENTRATION(a)

	C_f, mg/l			
C_o, mg/l	1.0	0.1	0.01	0.001
10	128.0	802.9	4612.8	10,000
1.0	--	73.0	457.1	2626.4
0.1	--	--	41.7	260.3
0.01	--	--	--	23.7

(a) Carbon doses in mg/l at neutral pH.

REMARKS: Solubility in H_2O is less than 0.0005 mg/l.

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BOTTOM OF IMAGE AREA; OUTSIDE DIMENSION FOR TABLES AND ILLUSTRATIONS

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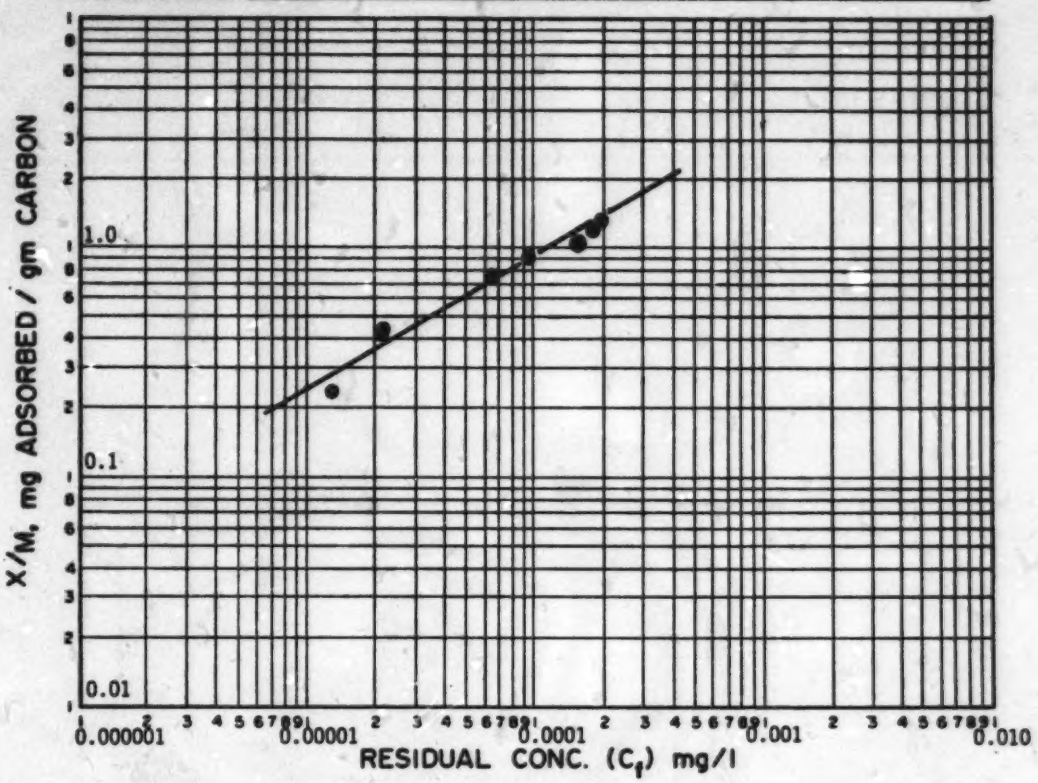
COMPOUND: Benzo(k)fluoranthene



DATE	Co mg/l[1]	mg/l Carbon	C_F mg/l	X / $C_o - C_F$	X/M mg/l	
1212B	0.000471	0.2817	0.000164	0.000307	1.090	
1313C[2]	0.000365	1.4930	0.000013	0.000352	0.236	
1213A*	0.000397	0.1420	0.000194	0.000203	1.430	
1213B	0.000377	0.1462	0.000182	0.000195	1.336	
1217A*	0.000349	0.7396	0.000021	0.000328	0.443	
1217B	0.000337	0.7463	0.000021	0.000316	0.423	
1319A**	0.000358	0.2915	0.000095	0.000263	0.902	
1219C	0.000391	0.4202	0.000065	0.000326	0.776	

*Solution pumped overnight
[1]Value after 2 hr of air stirring
**Solution pumped 42 hours
[2]Value after 16 hr of air stirring

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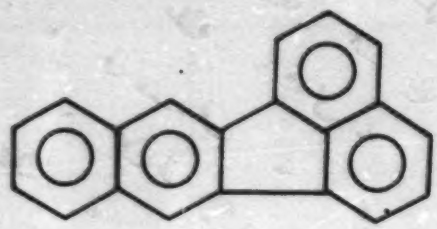
COMPOUND: Benzo(k)fluoranthene

STRUCTURE:



FORMULA: $C_{20}H_{12}$ **MOL. WT.** 252.32

FREUNDLICH PARAMETERS	pH		
	7.1		
K	183		
1/n	0.57		
Corr. Coef. r	0.98		
INITIAL CONC. mg/l	ADSORPTION CAPACITY, mg/gm		
10	683		
1.0	183		
0.1	48.9		
0.01	13.1		

CARBON DOSES REQUIRED TO ACHIEVE INDICATED CHANGE IN CONCENTRATION[a]

	C_f, mg/l			
C_o, mg/l	1.0	0.1	0.01	0.001
10	49.2	202.4	763.1	2854.2
1.0	--	18.4	75.6	285.2
0.1	--	--	6.9	28.3
0.01	--	--	--	2.6

(a) Carbon doses in mg/l at neutral pH.

REMARKS: Solubility in H_2O is less than 0.0005 mg/l.

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BOTTOM OF IMAGE AREA, OUTSIDE DIMENSION FOR TABLES AND ILLUSTRATIONS

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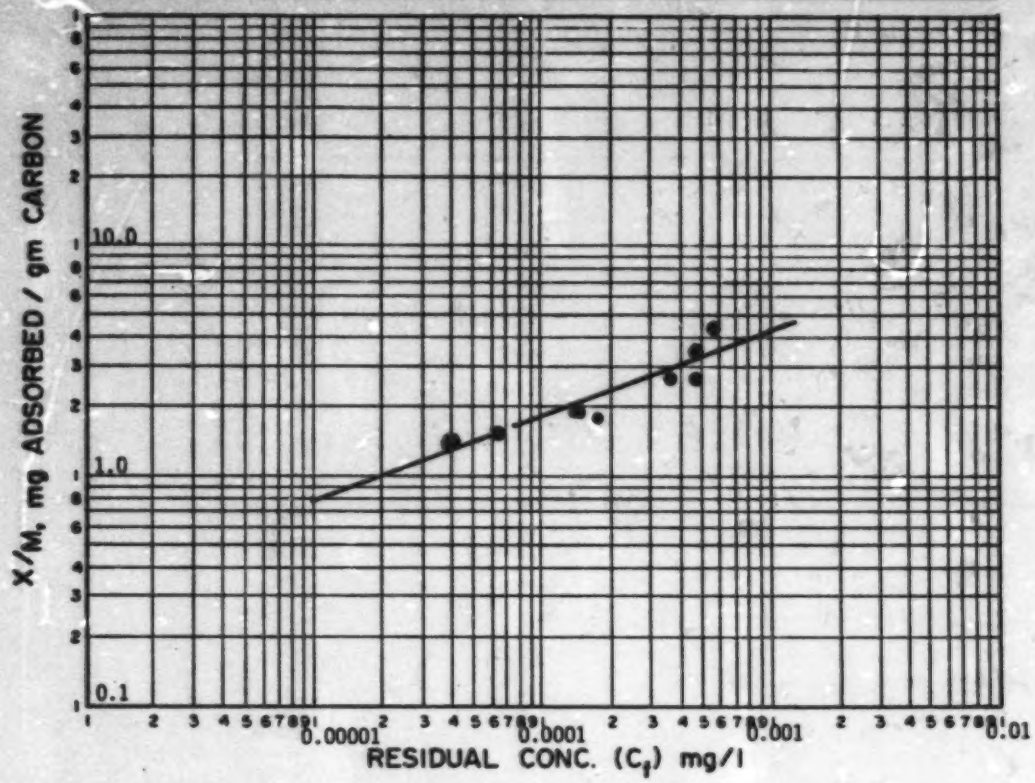
COMPOUND: 3,4-Benzofluoranthene/(B(b)F



DATE	M Carbon Dose mg/l	C_o ppm	C_f ppm	X C_o-Cf	X/M mg/g	
11/19 A*	0.725	0.00120	0.000064	0.001136	1.567	
11/19 B	0.806	0.00121	0.000040	0.001170	1.452	
11/20 A*	0.286	0.00129	0.000483	0.000807	2.822	
11/20 B	0.286	0.001184	0.000372	0.000812	2.839	
11/20 A'	0.143	0.00120	0.000583	0.000617	4.315	
11/21 B*	0.143	0.00100	0.000483	0.000517	3.615	
11/21 A	0.429	0.00100	0.000150	0.000850	1.981	
11/21 B'	0.429	0.00100	0.000172	0.000828	1.930	

*Had been pumped for 16 hours.

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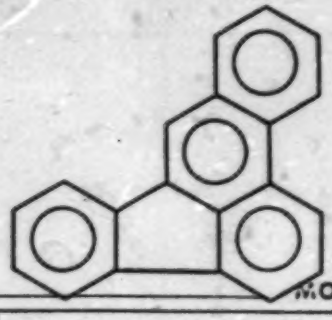
COMPOUND: 3,4-Benzofluoranthene/B(b)F

STRUCTURE:



FORMULA: $C_{20}H_{12}$ **MOL. WT.** 252.32

FREUNDLICH PARAMETERS	pH		
	7.0		
K	55.5		
1/n	0.37		
Corr. Coef. r	0.95		
INITIAL CONC. mg/l	ADSORPTION CAPACITY, mg/gm		
0.001	4.29		
0.0001	1.83		
0.00001	0.780		
0.000001	0.332		

CARBON DOSES REQUIRED TO ACHIEVE INDICATED CHANGE IN CONCENTRATION(a)

	C_f, mg/l			
Co, mg/l	0.0001	0.00001	0.000001	0.0000001
0.001	0.492	1.269	3.006	7.061
0.0001	--	0.115	0.298	0.705
0.00001	--	--	0.027	0.070
0.000001	--	--	--	0.006

(a) Carbon doses in mg/l at neutral pH.

REMARKS: Solubility approximately 0.0012 mg/l.

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BOTTOM OF IMAGE AREA, OUTSIDE DIMENSION FOR TABLES AND ILLUSTRATIONS

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APPENDIX B

PREPARATION OF NUTRIENT FOR STATIC AND CONTINUOUS BIODEGRADATION TESTS

NUTRIENT

Reagents

1. Distilled water.
2. Calcium choloride solution:
   Dissolve 27.5 g anhydrous $CaCl_2$ in distilled water and dilute to 1 l.
3. Magnesium sulfate solution:
   Dissolve 22.5 g $MgSO_4 \cdot 7H_2O$ in distilled water and dilute to 1 l.
4. Ferric chloride solution:
   Dissolve 0.25 g $FeCl_3 \cdot 6H_2O$ in distilled water and dilute to 1 l. These solutions should be stored in the dark, preferably in a refrigerator, and discarded at the first sign of turbidity.
5. Phosphate buffer solution:
   Dissolve 8.5 g potassium dihydrogen phosphate, KH_2PO_4, 21.75 g dipotassium hydrogen phosphate, K_2HPO_4, 33.4 g disodium hydrogen phosphate heptahydrate, $Na_2HPO_4 \cdot 7H_2O$, and 1.7 g ammonium chloride, NH_4Cl, in about 500 ml distilled water and dilute to 1 l.

Preparation of Medium

For each liter of distilled water add 1 ml of each of the following solutions in the order indicated, mixing after each addition:

1. calcium chloride solution
2. magnesium sulfate solution
3. ferric chloride solution
4. phosphate buffer solution

Weigh 0.055 g of yeast extract (Difco or equivalent), add to 1 l of the above solution and dissolve. Dispense 90 ml of medium into 250-ml Erlenmeyer flasks. The medium should be used within 3 hours after preparation unless sterilized. If an autoclave is available, larger batches of medium may be prepared, dispensed in flasks, and sterilized at 121°C for 15 min.

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When this is done, sufficient additional distilled water must be added to the medium to offset the sterilization loss. The flasks should be stopperred with cotton and capped with aluminum foil before sterilization. Foil caps and cotton stoppers are used to retard evaporation and maintain sterility until the medium is used. They are removed during the test.

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PREPARATION OF MINERALIZED WATER FOR CARBON ADSORPTION AND OZONATION TESTS

MINERALIZED WATER

Stock Solutions

1. Dissolve 21.96 g potassium dihydrogen phosphate, KH_2PO_4, in 1 l H_2O.
2. Dissolve 128.38 g magnesium sulfate heptahydrate, $MgSO_4 \cdot 7H_2O$, in 1 l H_2O.
3. Dissolve 273.7 g calcium chloride hexahydrate, $CaCl_2 \cdot 6H_2O$, in 1 l H_2O.

6-1/2"

Preparation

Use 95 ml of each stock medium in 45 l of water. Adjust the pH from the 6.8 of the stock solution to 7.5 by adding 16.8 g of sodium bicarbonate, $NaHCO_3$.

ANALYSIS OF BENZIDINE PLUS 3,3'-DICHLOROBENZIDINE

A survey of the literature revealed that several methods have been reported for the analysis of benzidine in different matrices. The procedure of Nony and Bowman was chosen. It involves solvent extraction of the sample with benzene, drying and concentration using a K-D evaporator, derivatization and analysis by gas chromatography-flame ionization detection. The method is described below. The EPA Chloramine T complexation method was not considered because of the possible formation of interferences in the treatment experiments. The procedure of Nony and Bowman was found to be satisfactory (Nony, C.R., and M.C. Bowman. Carcinogens and Analogs: Trace Analysis of Thirteen Compounds in Admixture in Wastewater and Human Urine. *Intern. J. Environ. Anal. Chem.* (submitted for publication). A benzidine recovery of 86.5 percent, ± 6.4 percent, was obtained in the analysis of duplicate samples spiked at the ppm level. In several cases, however, the samples were not completed derivatized for some reason. Similar problems were encountered by F.K. Kawahara of EPA's Environmental Monitoring and Support Laboratory, Cincinnati, Ohio, who is also using the Nony and Bowman procedure for the analysis of benzidine in wastewater. Some additional development work will be required before this procedure can be used for routine analysis.

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